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Determination of Particulate Matter and Alkaloids (as Nicotine) in Cigarette Smoke

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A collaborative smoking study for the determination of particulate matter and nicotine deliveries of cigarettes, by a method chosen by the Analytical Methods Committee of the Tobacco Chemists' Conference, was completed during the year. The 12 collaborators obtained coefficients of variation within laboratories of 4% and between laboratories of 10%. Each collaborator was asked to smoke 40 cigarettes (8 samples of 5 cigarettes). Non-filter (85 mm) cigarettes and filter (85 mm) cigarettes were analyzed. The cigarettes were conditioned for 24 hours at 75°F and 60% r.h. prior to selection for smoking. Cigarettes weighing within 20 mg of the average cigarette weight (50 randomly selected cigarettes) were marked to a 30 mm butt length. The cigarettes were smoked into a Cambridge filter holder by an automatic smoking machine which drew a 35 ml puff of 2-second duration once every minute. Five weight-selected cigarettes, marked to 30 mm butt, were smoked per Cambridge filter and the particulate matter (wet) was determined as the weight gain of the Cambridge filter. Nicotine delivery was determined by distilling the Cambridge filter pads and measuring the nicotine spectrophotometrically.

A collaborative smoking study for the determination of particulate matter (PM)[1] and alkaloids (as nicotine) in cigarette smoke was completed during the year. The Analytical Methods Committee of the Tobacco Chemists' Conference chose the method to be tested. The proposed, rapid method is a compromise of the various procedures now in use by the tobacco companies and related industries engaged in the analysis of cigarette smoke. The committee members believe the method, although empirical, is as reliable and as expeditious as can be prepared at the present time.

One carton of two different cigarettes, King Non-filter cigarettes and King Filter cigarettes, made for this study, was sent to each collaborator with a copy of the proposed method. Each collaborator was asked to smoke 40 cigarettes instead of 20 for this study. In addition to particulate matter (PM) and nicotine deliveries, cigarette total puff count was determined for each brand. No correction was made for moisture contained in the particulate matter.

METHOD

Sample Selection

Remove cigarettes from 5 randomly selected packages, place them in a tray or wire basket, and condition them for 24 hours at 75 ± 2°F and 60 ± 2% relative humidity (60% relative humidity can be obtained by placing a solution containing 26% water and 74% absolute glycerine by weight (ref. index = 1.437 and 20°C) in a closed desiccator). Determine average weight of 50 randomly selected, conditioned cigarettes. Select 20 cigarettes weighing within ± 20 mg of average weight. Cigarettes must not contain soft spots nor be loosely packed or frayed at either end. Mark each cigarette 30 mm from butt end with soft lead pencil or pen without puncturing paper. Store selected cigarettes at 75°F and 60% r.h. until they are to be smoked. If sample selection is made in room not maintained at 75 ± 2°C and 60 ± 2% r.h., cigarettes must be reconditioned for 4 hours before smoking. It is recommended that cigarette sample selection and the cigarette smok-

[1] Particulate Matter (PM) is arbitrarily defined as that material which is collected on the Cambridge CM113A filter under the prescribed smoking conditions.

ing be carried out in a laboratory room maintained at 75 ± 2°F and 60 ± 2% r.h.).

Smoking Machine Characteristics

Smoking machines must be automatic and capable of drawing puffs according to following specifications:

Puff volume.—35 ± 0.5 ml measured as volume of smoke that will be drawn from cigarette under actual machine smoking conditions. Puff volume should be checked before and after each smoking run with smoke collection trap in system. Puff volume may be measured by water-filled 50 ml buret and leveling bulb. Water levels in buret and bulb should be equal at start and finish, and as nearly equal during puff as possible.

Puff duration.—2 ± 0.2 sec. measured at cigarette under actual machine smoking conditions by hot wire anemometer or soap bubble manometer.

Puff frequency.—1 puff per 60 ± 1 sec.

Cigarettes must be free from drafts (other than normal convection) while being smoked.

Particulate Matter

Apparatus

(a) *Filter holder.*—A Lucite (or aluminum) filter holder consisting of threaded inner and outer parts and Teflon gasket.

(b) *Filter disc.*—Cut discs 1.74" (44 mm) in diameter from CM113A fiber glass sheet made by the Cambridge Filter Corp., 738 Erie Blvd., East Syracuse 3, N.Y.,[2] or equivalent filter material. Filters must collect at least 99.9% of all particles 0.3 μ in diameter and over at a flow rate of 28 linear ft per min., have a maximum pressure drop not exceeding 93 mm water at 28 ft per min., and contain not more than 5% acrylic-type binder.

(c) *Lucite guide.*—A Lucite guide is used to assist in placing rubber membrane on filter holder. (The holder, guide, and filter medium, CM113A, both in sheets and as 1.74" diameter discs, are obtainable from Phipps and Bird, Inc., Richmond, Va.[2])

(d) *Rubber membrane.*—Cut square piece of medium latex dental dam, approximately 3.5 × 3.5 cm. Place between two pieces of rubber tile, or other suitable material, and punch hole 4–6 mm in diameter in center of sheet with cork borer of appropriate size; size of hole depends on circumference of cigarettes. Latex rubber sleeves, 8 mm in diameter and 20 mm long, may also be used.

(e) *Rubber "O" ring.*—"O" ring, 3/8" i.d., may be made by slicing off thin section of 3/8" i.d. rubber tubing or may be purchased from Linear, Inc., State Rd. & Levick St., Philadelphia, Pa.[2]

(f) *Cambridge filter assembly.*—For a detailed description of smoking apparatus see Wartman, W. B., Coghill, E. C., and Barlow, E. S., *Anal. Chem.*, 31, 1705 (1959).

Determination

Using Lucite guide, place rubber membrane on filter holder by inserting offset end of Lucite guide through hole in rubber membrane and then into entrance tube of filter holder; holding guide and membrane firmly against filter holder, roll rubber "O" ring over guide and into position around membrane and in groove on entrance tube. It may sometimes be necessary to center aperture and adjust its diameter by manipulating rubber membrane. Trim excess rubber membrane with scissors. Fit glass fiber filter disc into filter holder with rough side toward port through which cigarette is inserted. Position Teflon gasket with flat side resting against filter disc, and screw in Lucite plug securely against gasket. Tighten with special wire wrench with ends fitted into two sockets on back of plug.

Wipe gently with soft cloth or tissue and weigh filter assembly to nearest 0.2 mg. Connect filter assembly to smoking machine by short piece of rubber or other suitable tubing with heavy enough wall so that cigarette and filter assembly will be held in horizontal position. Keep volume between filter holder and machine to minimum. (Do not use surge flask in smoking machine assembly.) Test smoking apparatus and filter assembly for leaks. Insert cigarette through hole in rubber membrane until end of butt is approximately flush with inner end of holder tube, i.e., to depth of about 7/16". Take care that butt end does not come in contact with filter disc. Withdraw cigarette slightly so that lip of orifice in rubber membrane projects outward and forms a snug-fitting collar without crimping or pinching cigarette. Occasionally, it may be necessary to shift position of cigarette slightly in or out, to insure that collar surrounds smooth portion of cigarette and provides leak-free seal.

Light cigarette at beginning of first puff (an electric coil lighter is suggested). Smoke each

[2] *Mention of brand or firm name does not constitute endorsement by the Department of Agriculture over others of a similar nature not mentioned.*

cigarette until burning coal reaches 30 mm mark. If operator anticipates that coal will reach 30 mm mark during puff, he should use judgment whether to allow cigarettes to burn beyond mark or to stop smoking it short of mark. In the smoking of cigarettes, "overs" should be balanced with "unders" for each filter. After last puff, let cigarette remain in holder, free-burning, until few seconds before next puff will be drawn by machine; then quickly remove butt from holder and allow clearing puff of air to draw in smoke remaining in entrance port of assembly. In same manner, smoke total of 5 cigarettes through filter. Immediately after 5 cigarettes have been smoked, disconnect filter assembly from apparatus, wipe, and weigh to the nearest 0.2 mg.

Record gain in weight of filter assembly. (Save smoke samples for "nicotine" analysis.) Calculate particulate matter (wet) by:

wt PM (wet)/cigarette = gain in wt of filter assembly (mg)/5.

Smoke 4 samples of 5 cigarettes each and average results.

Alkaloids (As Nicotine)—Modified Kjeldahl Still

Reagent and Apparatus

(a) *Sodium hydroxide-sodium chloride solution.*—NaOH solution, 30% by weight, saturated with NaCl.

(b) *Steam distillation apparatus.*—Kjeldahl flask, 500 ml, fitted with steam tube, trap, and condenser.

(c) *Spectrophotometer.* — Beckman Model DU or other instrument capable of accurately measuring absorbance in 200–300 mμ range and having slit width not greater than 5 mμ.

Determination

Transfer filter disc containing particulate matter to Kjeldahl flask and add 50 ml 0.1*N* HCl. Wipe out entrance chamber of filter holder with two small swabs of cotton or pieces of CM113A filter held in forceps, and add them to flask. Fit flask for steam distillation with steam inlet tube, spray trap, and condenser. Steam-distill acid solution for 10–15 min., keeping volume approximately constant by applying more heat. Discard condensate. Stop steam distillation, place 500 ml volumetric flask containing 25 ml (1 + 11) HCl under condenser with condenser tip dipping into acid solution, add 25 ml NaOH-NaCl solution to distillation flask, and connect immediately. Keeping volume in distilling flask between 75 and 100 ml, rapidly steam-distill until volume of distillate is about 450 ml; then add water to mark and mix. Determine absorbance of distillate at 236, 259, and 282 mμ against blank of 0.05*N* HCl, using 1 cm cells. Calculate total weight of "nicotine" in smoke sample as follows:

A'_{259} = absorbance of "nicotine" corrected for background = $1.059 \left| A_{259} - \frac{1}{2} (A_{236} + A_{282}) \right|$

Total wt "nicotine" (mg) = $(A'_{259} \times 500)/(a \times b)$

where a is absorptivity of nicotine in 0.05*N* HCl solution and b is cell length.

$a = A/(c \times b)$, where A is absorbance at 259 mμ and c is concentration in g/L of standard solution of pure nicotine in 0.05*N* HCl. Purify nicotine by repeated distillation until physical constants reach constant values which agree with those for pure nicotine.

Wt "nicotine" (mg)/cigarette = total wt "nicotine" (mg)/5.

Make separate analysis on each filter, and average results.

Alkaloids (As Nicotine)—Griffith Still

Apparatus

Steam distillation apparatus. — Griffith still (see "The Rapid Determination of Total Alkaloids by Steam Distillations," by R. B. Griffith, *Tobacco Sci.*, 1, 130 (1957)). May be obtained from Consolidated Glass Works, Inc., Kingsport, Tenn.

Determination

Transfer filter disc containing particulate matter to Griffith still and add 5 ml 0.2*N* HCl. Wipe out entrance port of each filter with two small cotton swabs or pieces of CM113A filter held in forceps, and add them to flask. Rapidly steam-distill acid solution, collecting about 100 ml distillate, keeping volume approximately constant. Discard condensate. Turn off steam, place 250 ml volumetric flask containing 10 ml HCl (1 + 9) under condenser with condenser tip dipping into acid solution, and add 5 ml NaOH-NaCl solution, reagent (a) of previous method. Keeping volume in flask approximately constant, rapidly distill about 225 ml, add water to mark, and mix. Proceed as for modified Kjeldahl still, beginning "Determine absorbance of distillate . . ." (Factor for calculation of total wt "nicotine" (mg): use × 250 instead of × 500.)

Table 1. Average weight of equilibrated cigarettes (85 mm) and weight of tobacco burned (30 mm butt), grams

Coll.	Non-Filter		Filter	
	Cigarette Weight	Tobacco Burned	Cigarette Weight	Tobacco Burned
1	1.195	0.766	1.106	0.769
2	1.208	0.759	1.125	0.768
3	1.196	0.759	1.116	0.763
5	1.202	0.780	1.114	0.786
8	1.178	0.768	1.090	0.761
9	1.203	0.781	1.124	0.769
11	1.196	0.774	1.115	0.774
13	1.210	0.775	1.123	0.763
20	1.196	0.797	1.113	0.801
22	1.197	0.769	1.117	0.765
23	1.214	0.780	1.112	0.780
24	1.231	0.801	1.124	0.788
Mean	1.202	0.775	1.115	0.774
Std dev.	0.013	0.015	0.010	0.012
Coeff. of var., %	1.1	1.9	0.9	1.6

Results

The collaborators were asked to determine the average weight of each cigarette type, after it had been conditioned for 24 hours at 75 ± 2°F and 60 ± 2% relative humidity, and the average weight of tobacco burned (obtained by cutting off the 30 mm cigarette butt and weighing the remaining cigarette rods including the cigarette paper).

Table 1 shows average cigarette weight, average weight of tobacco burned, the mean weights of two cigarettes, the interlaboratory standard deviations, and the coefficient of variation in per cent as obtained by the twelve collaborators. As would be expected, cutting off the 30 mm cigarette butt resulted in a higher coefficient of variation in the weight of tobacco burned than that obtained for the cigarette weight; i.e., 1.6 and 1.9 versus 0.9 and 1.1, respectively.

The collaborators were instructed to smoke eight ports (samples) of each brand, a total of forty cigarettes per brand. The average

Table 2. Average total number of puffs per five cigarettes (85 mm)

Coll.	Non-Filter				Filter			
	No. of Puffs		Std Dev.		No. of Puffs		Std Dev.	
	8 ports	4 ports	8 ports	4 ports	8 ports	4 ports	8 ports	4 ports
1	59	59	0.5	0.6	53	53	0.7	0.8
2	48	48	1.5	1.7	48	49	1.6	1.6
3	52	52	1.3	0.6	50	50	0.0	0.0
5	49	50	1.0	1.0	47	48	1.7	1.7
8	46	46	0.9	0.8	45	45	0.4	0.0
9	51	50	2.2	1.4	49	49	1.4	1.9
11	50	50	0.4	0.0	50	50	0.0	0.0
13	50	49	1.1	0.8	48	48	1.6	1.0
20	50	50	1.8	2.2	50	50	1.4	1.7
22	55	55	1.4	1.1	53	54	1.4	1.1
23	55	55	0.5	0.6	52	52	1.3	1.5
24	51	49	2.0	1.0	48	49	0.5	0.6
Mean	51.3	51.0	1.31[a]	1.13[a]	49.5	49.6	1.16[a]	1.22[a]
Coeff. of var., %								
Within			2.6	2.2			2.3	2.5
Between			6.9	6.9			5.0	4.8
Mean std dev. between laboratories			3.52	3.54			2.47	2.38

[a] Mean standard deviation within laboratories.

Table 3. Particulate matter delivery per cigarette, mg

Coll.	Non-Filter				Filter			
	Particulate Matter		Std Dev.		Particulate Matter		Std Dev.	
	8 ports	4 ports	8 ports	4 ports	8 ports	4 ports	8 ports	4 ports
1	26.0	26.2	0.64	0.91	22.8	22.5	0.59	0.69
2	30.4	30.8	1.35	1.78	23.8	23.8	1.01	0.25
3	31.7	31.8	0.75	0.62	24.8	25.1	0.55	0.22
5	30.4	30.6	0.87	0.99	2[illegible].3	[illegible].0	1.14	1.12
8	28.9	29.6	1.71	1.08	21.2	[illegible].9	0.72	0.61
9	33.0	32.8	1.13	0.13	24.3	3.8	0.94	1.03
11	33.6	33.4	0.61	0.73	25.7	25.5	0.64	0.49
13	31.8	31.7	0.39	0.22	23.3	23.2	0.42	0.48
20	31.9	32.2	0.67	0.73	24.0	24.3	0.83	0.77
22	37.4	37.9	1.78	2.27	28.6	28.5	0.31	0.40
23	37.1	37.3	1.11	1.57	29.6	29.7	1.31	1.47
24	33.9	33.2	0.95	0.14	25.1	25.3	0.43	0.54
Mean	32.2	32.3	1.08[a]	1.13[a]	24.6	24.6	0.80[a]	0.76[a]
Coeff. of var., %								
Within			3.4	3.5			3.2	3.1
Between			9.9	9.8			10.0	10.0
Mean std dev. between laboratories			3.19	3.15			2.45	2.48

[a] Mean standard deviation within laboratories.

total number of puffs per five cigarettes and the standard deviation both for the first four of the eight ports and for the eight ports (samples of five cigarettes) as obtained by the twelve collaborators are shown in Table 2. The mean coefficients of variation within and between the laboratories are also shown in this table. The variation in the total puff count was higher for the non-filter cigarette than for the filter cigarette. The between-laboratories variation was twice that obtained within laboratories. The particulate matter and nicotine deliveries (wet) for the two cigarette brands are shown in Tables 3 and 4. The standard deviations obtained by each collaborator for the first four of the eight ports and for all eight ports are included in these tables, together with the mean coefficients of variation within and between laboratories. The coefficients of variation between laboratories for particulate matter was about 3 times and for nicotine about 2 times that within laboratories. Furthermore, the collaborators did not obtain a higher degree of precision by increasing the number of samples (ports of five cigarettes) from four to eight. Thus, the prescribed procedure of four samples per cigarette brand is satisfactory.

Discussion

The coefficients of variation obtained within the twelve collaborators on total puff count (eight samples), particulate matter (four and eight samples), and nicotine deliveries were less than 5%. This is a reasonable value when the nature of the sample and number and type of variables are considered. However, the coefficients of variation obtained between the twelve collaborators for particulate matter and nicotine were approximately 10%. Inspection of the data shows that the particulate matter and nicotine deliveries obtained by two of the collaborators were significantly lower than the averages of the collaborators, and two

Table 4. Nicotine delivery per cigarette, mg

Coll.	Non-Filter				Filter			
	Nicotine		Std Dev.		Nicotine		Std Dev.	
	8 ports	4 ports	8 ports	4 ports	8 ports	4 ports	8 ports	4 ports
1	1.24	1.24	0.07	0.06	1.11	1.12	0.05	0.07
2	1.47	1.44	0.08	0.10	1.22	1.22	0.04	0.01
3	1.63	1.60	0.05	0.04	1.29	1.30	0.03	0.02
5	1.51	1.49	0.05	0.07	1.22	1.21	0.08	0.12
8	1.57	1.59	0.09	0.06	1.19	1.17	0.03	0.02
9	1.62	1.63	0.09	0.13	1.28	1.27	0.05	0.07
11	1.65	1.62	0.05	0.04	1.34	1.32	0.06	0.08
13	1.55	1.55	0.02	0.03	1.18	1.18	0.02	0.03
20	1.47	1.43	0.06	0.04	1.17	1.17	0.03	0.03
22	1.73	1.75	0.04	0.03	1.41	1.41	0.03	0.03
23	1.80	1.81	0.06	0.08	1.47	1.51	0.09	0.05
24	1.59	1.59	0.04	0.05	1.23	1.20	0.07	0.08
Mean	1.57	1.56	0.061[a]	0.066[a]	1.26	1.26	0.054[a]	0.069[a]
Coeff. of var., % Within			3.9	4.2			4.3	4.8
Between			9.1	9.6			8.2	9.0
Mean std dev. between laboratories			0.143	0.149			0.103	0.111

[a] Mean standard deviation within laboratories.

Table 5. Total puff count, particulate matter, and nicotine deliveries of cigarettes for eight collaborators[a], four ports

Variation	Non-Filter			Filter		
	mg/ Cigt.	Std Dev.	Coeff. Var., %	mg/ Cigt.	Std Dev.	Coeff. Var., %
			Total Puff Count			
Within labs.	49.7	1.26	2.5	49.0	1.31	2.7
Between labs.	49.7	0.95	1.9	49.0	0.80	1.6
			Particulate Matter			
Within labs.	32.0	0.84	2.6	24.2	0.69	2.8
Between labs.	32.0	1.04	3.2	24.2	1.00	4.1
			Nicotine			
Within labs.	1.54	0.071	4.6	1.23	0.066	5.4
Between labs.	1.54	0.081	5.2	1.23	0.055	4.5

[a] Omitting Collaborators 1, 8, 22, and 23.

collaborators' results were significantly higher than the averages. Three of these four laboratories reported puff counts significantly higher than the remaining laboratories, which would indicate differences in their smoking machines. The remaining labora-

Table 6. Summary of per cent coefficient of variation within and between laboratories

Variable	Within Laboratories		Between Laboratories	
	Non-Filter	Filter	Non-Filter	Filter
Twelve Collaborating Laboratories				
Cigarette weight	—	—	1.1	0.9
Tobacco burned	—	—	1.9	1.6
Total puff count	2.6	2.3	6.9	5.0
Particulate matter				
(8 ports)	3.4	3.2	9.9	10.0
(4 ports)	3.5	3.1	9.8	10.0
Nicotine				
(8 ports)	3.9	4.3	9.1	8.2
(4 ports)	4.2	4.8	9.6	9.0
Omitting Laboratories 1, 8, 22, and 23				
Total puff count				
(4 ports)	2.5	2.7	1.9	1.6
Particulate matter				
(4 ports)	2.6	2.8	3.2	4.1
Nicotine				
(4 ports)	4.6	5.4	5.2	4.5

tory with different results had cigarettes which weighed significantly less than the cigarette averages obtained by the twelve collaborators. This collaborator also reported puff counts which were significantly lower than the average puff counts. Therefore, we believe that we are justified in recalculating the data without Collaborators 1, 8, 22, and 23. Collaborator 1's smoking machine definitely is different from the others; he obtained the highest puff count and among the lowest particulate matter and nicotine deliveries. Collaborator 8's cigarettes apparently were drier than the other collaborators' cigarettes; they burned faster and delivered less particulate matter than the average obtained by the collaborators. Collaborators 22 and 23's smoking machines appear to be different from those of the other collaborators; they obtained high puff counts and significantly higher particulate matter and nicotine deliveries than did the other collaborators.

The average values obtained by the eight collaborators are not different from the average values obtained by the twelve collaborators (Table 5). The coefficients of variation between the eight collaborators, however, are less than 5% for particulate matter delivery and approximately 5% for nicotine. The coefficients of variation are summarized in Table 6.

Recommendation

It is recommended that the proposed cigarette smoking method for the determination of particulate matter and alkaloids (as nicotine) in cigarette smoke be further studied and that studies be initiated to develop a method for the determination of total condensables in cigarette smoke.

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The recommendation of the Associate Referee was approved by the General Referee and by Subcommittee A, and was accepted by the Association. See *This Journal*, 47, 122 (1964).